Supplementary Information

Chronic exposure to hexavalent chromium induces esophageal tumorigenesis via activating the Notch signaling pathway

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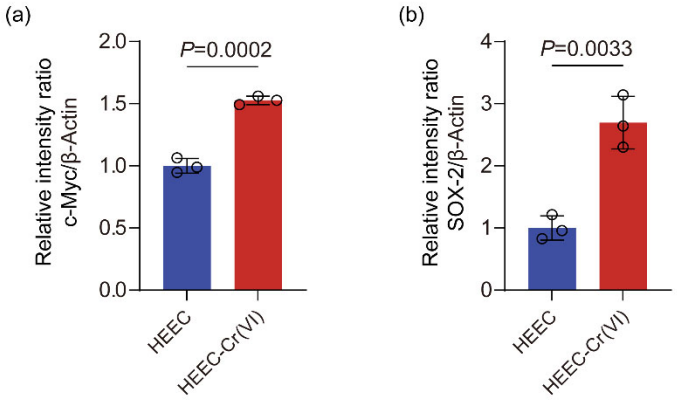
Supplementary information included in this PDF file:

Supplementary Figures 1 to 9

Supplementary Tables 1 and 2

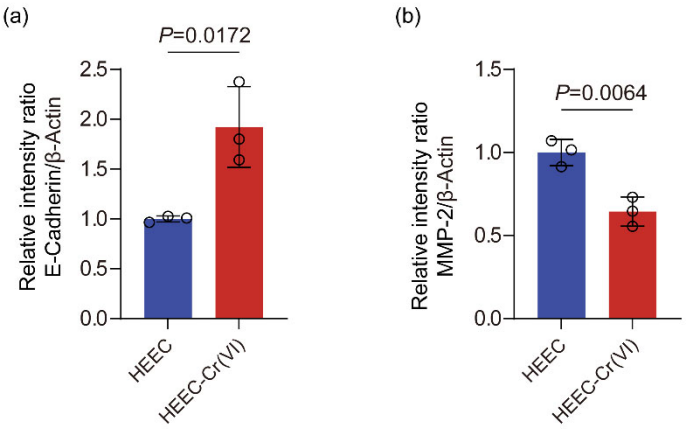
Fig. S1



Supplementary Figure 1. The quantitative analysis of western blot for c-Myc and SOX-2 proteins in HEEC and HEEC-Cr(VI) cells.

(a, b) The histograms illustrate the normalized expression levels of the c-Myc (a) and SOX-2 (b) in HEEC and HEEC-Cr(VI) cells from three replicate experiments. Statistical significance was determined using an unpaired *t*-test.

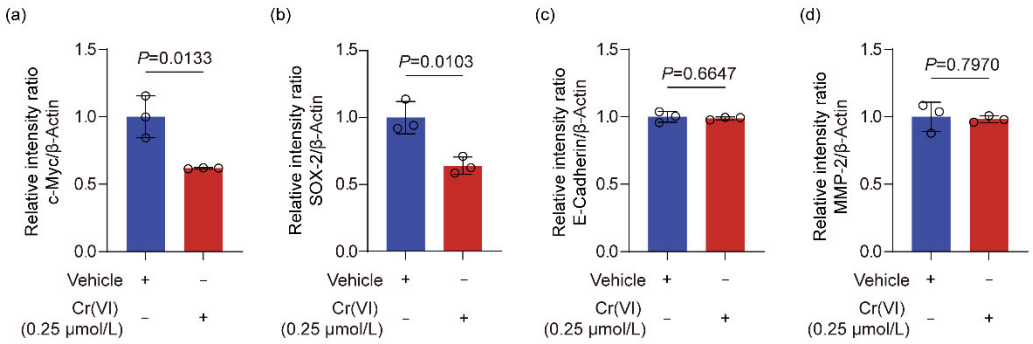
Fig. S2



Supplementary Figure 2. Quantitative comparison of E-Cadherin and MMP-2 expression in HEEC and HEEC-Cr(VI) cells.

(a, b) The levels of E-Cadherin (a) and MMP-2 proteins (b) in HEEC and HEEC-Cr(VI) cells from three independent experiments are quantified using the ImageJ software. Statistical significance was assessed by performing an unpaired *t*-test.

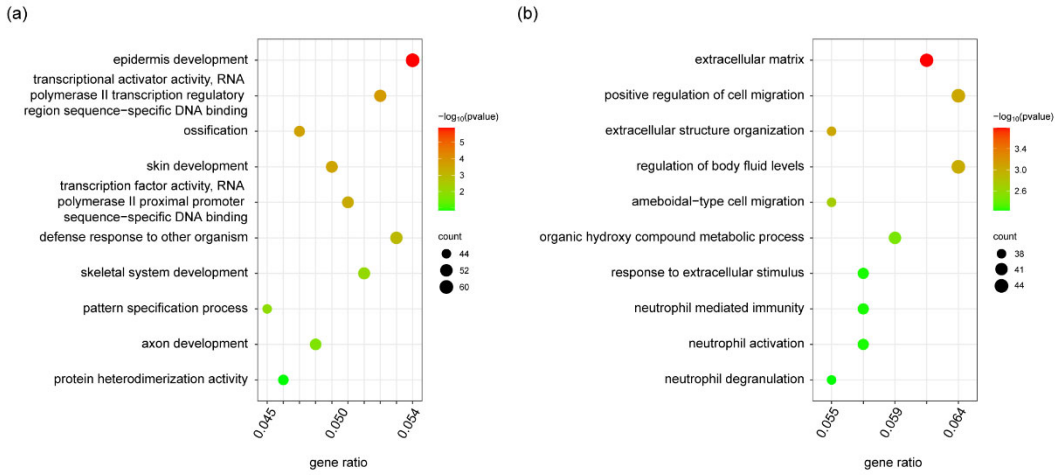
Fig. S3



Supplementary Figure 3. Assessment of protein expression levels between acute Cr(VI) exposure and vehicle groups.

(a-d) The histograms show the levels of c-Myc (a), SOX-2 (b), E-cadherin (c), and MMP-2 proteins (d) in HEEC cells treated with vehicle or Cr(VI) (0.25 μmol/L) for 48 hours. The data from three independent assays were displayed as mean±SD and statistically analyzed by an unpaired *t*-test.

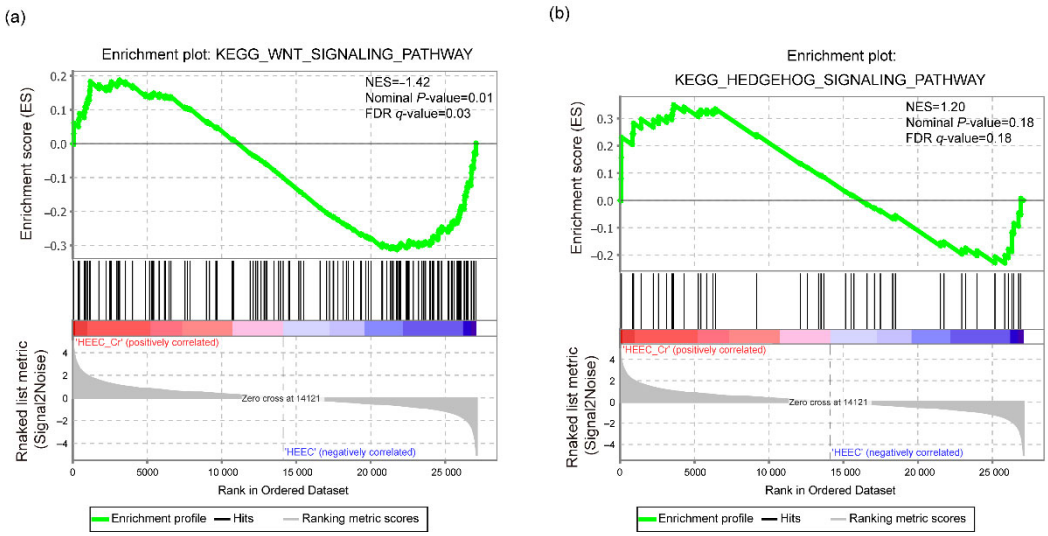
Fig. S4



Supplementary Figure 4. GO analysis based on DEGs identified in HEEC-Cr(VI) cells.

(a, b) Gene ontology (GO) enrichment analysis based on 1447 up-regulated (a) or 934 down-regulated DEGs (b) was performed to determine the top 10 enriched terms.

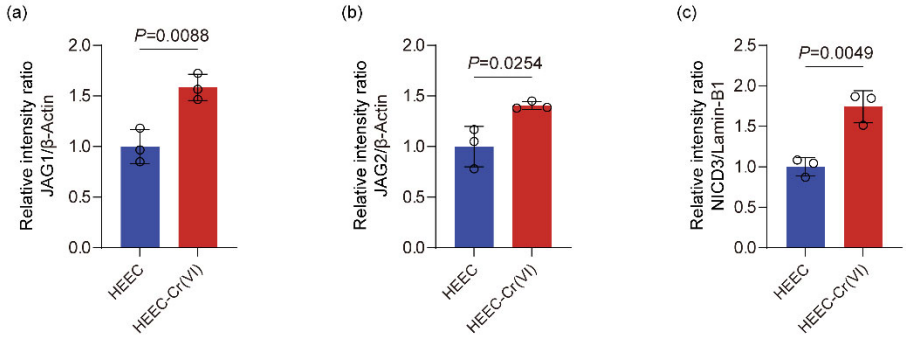
Fig. S5



Supplementary Figure 5. GSEA for Wnt and Hedgehog signaling according to RNA-Seq.

(a, b) GSEA was conducted to evaluate the status of the Wnt (a) and Hedgehog (b) signaling pathways based on RNA-Seq data from HEEC-Cr(VI) cells.

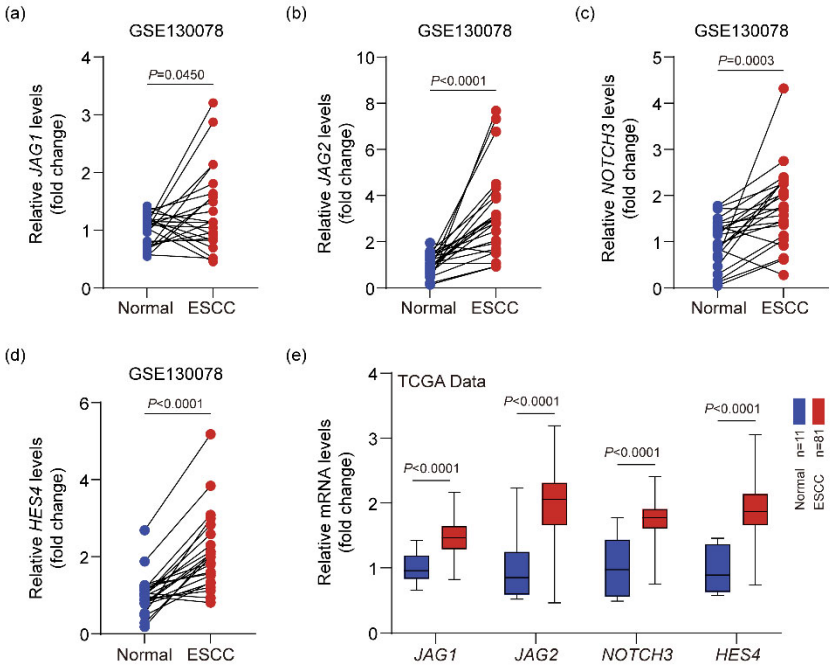
Fig. S6



Supplementary Figure 6. The quantitative analysis for JAG1, JAG2, and NICD3 proteins between HEEC and HEEC-Cr(VI) cells.

(a-c) The protein expression of JAG1 (a), JAG2 (b), and NICD3 (c) in HEEC and HEEC-Cr(VI) cells from three replicate experiments was quantified using the ImageJ software. An unpaired *t*-test was applied to evaluate the statistical significance of the differences.

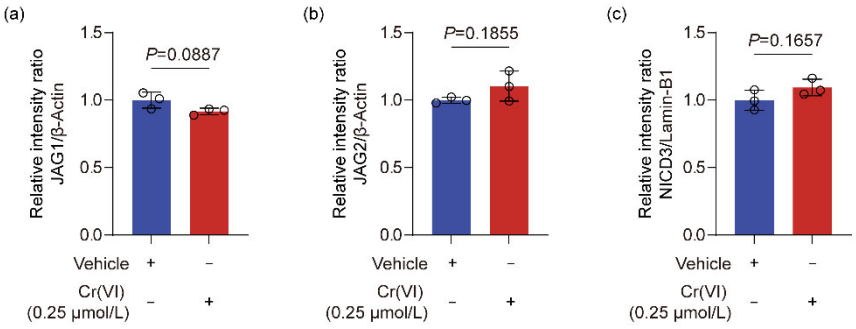
Fig. S7



Supplementary Figure 7. The mRNA levels of key components in the Notch pathway are upregulated in ESCC according to the GEO and TCGA databases.

(a-e) The mRNA levels of *JAG1*, *JAG2*, *NOTCH3*, and *HES4* in ESCC and normal esophageal tissues were analyzed based on GEO datasets (GSE130078) (23 pairs of ESCC tissues) (a-d) or TCGA data (Normal, *n*=11; ESCC, *n*=81) from the UCSC Xeno platform (e). A paired *t*-test was performed to determine statistical significance in (a-d), while an unpaired *t*-test was applied in (e).

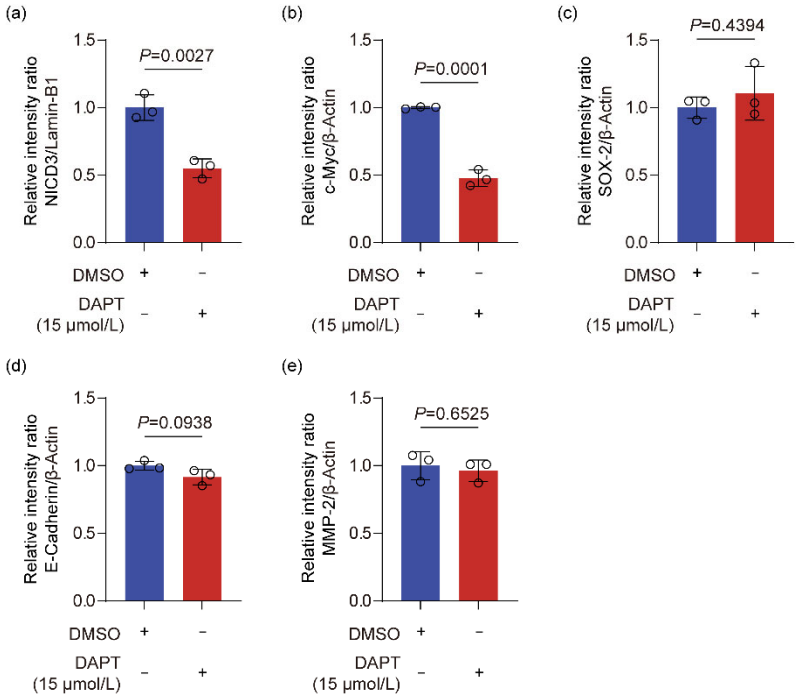
Fig. S8



Supplementary Figure 8. The quantitative comparison for JAG1, JAG2, and NICD3 proteins in HEEC cells with acute exposure or vehicle control.

(a-c) JAG1 (a), JAG2 (b), and NICD3 (c) protein levels in HEEC cells with acute exposure or vehicle control from three replicate experiments were analyzed by the ImageJ software. The statistical significance of the differences was evaluated using an unpaired *t*-test.

Fig. S9



Supplementary Figure 9. The comparison of target proteins' levels in HEEC-Cr(VI) cells treated with vehicle or DAPT.

(a-e) The histograms show quantification of the expression levels of NICD3 (a), c-Myc (b), SOX-2 (c), E-Cadherin (d), and MMP-2 (e) among groups. The data derived from three independent experiments are present as the mean±SD. Statistical significance was determined using the unpaired *t*-test.

Supplementary Table 1. The sequence of primers used in this study.

Target name	Sequence (5' to 3')	Application
JAG1	F: TGAGGCCGTTGCTGACTTAG R: TGAGATGCGGCACTCGATTT	qPCR
JAG2	F: TTCACCAAAGATCCTGGCCG R: TCATTGATGCTCCTGACCGC	qPCR
NOTCH3	F: TTCTTAGATCTTGGGGGCCT R: GGAAGAAGGAGGTCCCAGAC	qPCR
HES4	F: ATCCTGGAGATGACCGTGAG R: CGGTACTTGCCCAGAACG	qPCR
GAPDH	F: CATCACTGCCACCCAGAAGACTG R: ATGCCAGTGAGCTTCCCGTTCAG	qPCR

Supplementary Table 2. The information on antibodies used in this study.

Antibody Names	Source(catalog)	Application
Anti-E Cadherin antibody [EP700Y]	ABCAM (ab40772)	WB
MMP2 Polyclonal antibody	PROTEINTECH (10373-2-AP)	WB
Sox2 (D6D9) XP® Rabbit mAb	CST (#3579)	WB
c-Myc Antibody	CST (#9402)	WB
Jagged1 (D4Y1R) XP® Rabbit mAb	CST (#70109)	WB
Jagged2 antibody (F-4)	SANTA CRUZ (sc-515725)	WB, IF
Notch3 (D11B8) Rabbit mAb	CST (#5276)	WB
Notch 3 antibody (A-6)	SANTA CRUZ (sc-515825)	IF
GAPDH Monoclonal antibody	PROTEINTECH (60004-1-Ig)	WB
Lamin B1 Polyclonal antibody	PROTEINTECH (12987-1-AP)	WB
β-Actin Rabbit mAb (High Dilution)	ABCLONAL (AC026)	WB

Raw data for western blot

Fig1

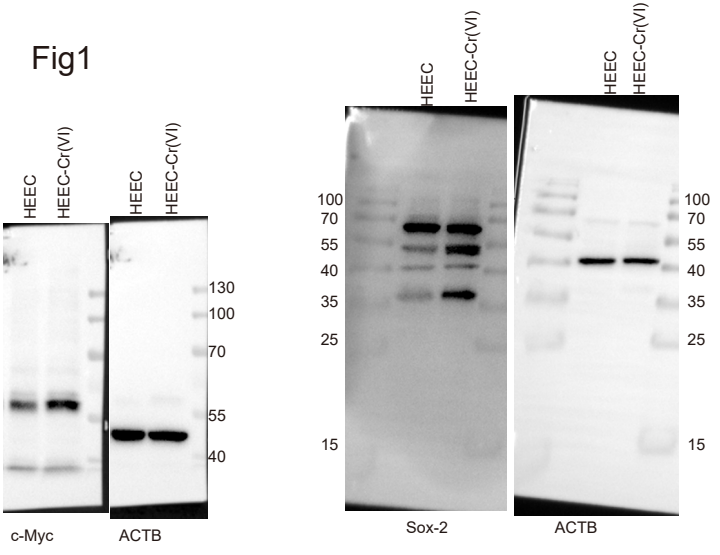


Fig3

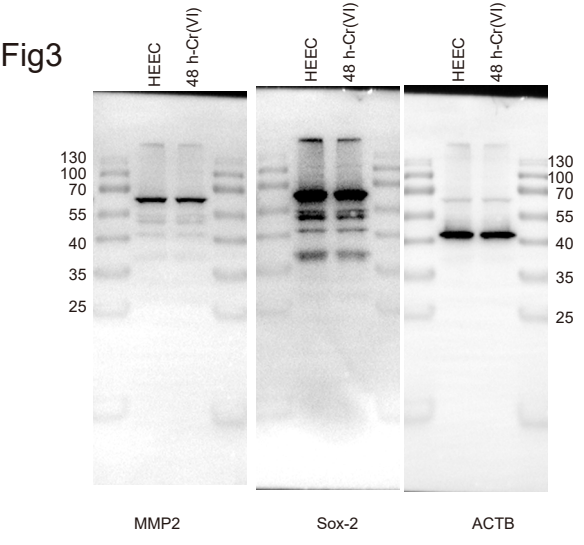


Fig2

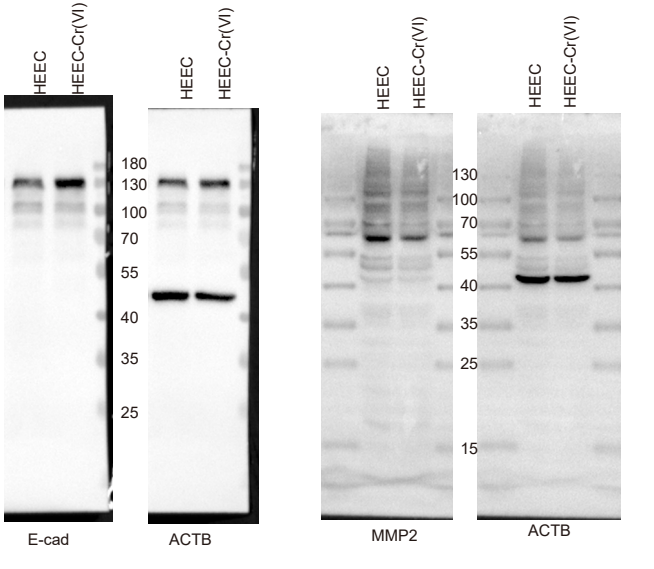


Fig3

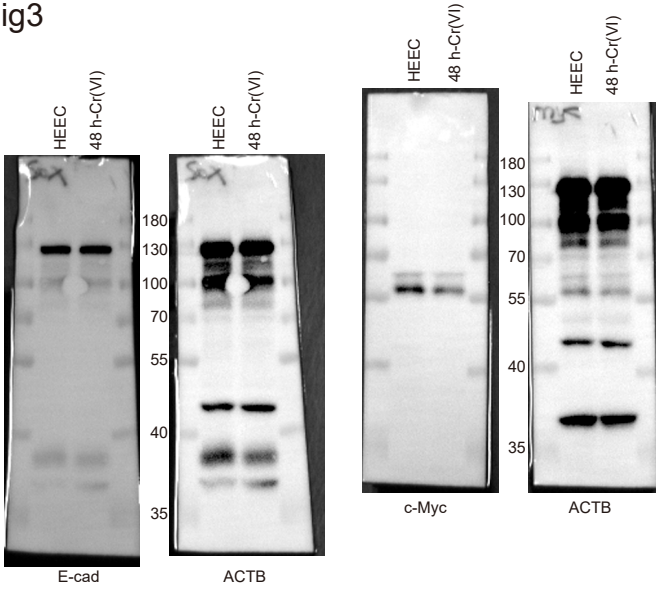
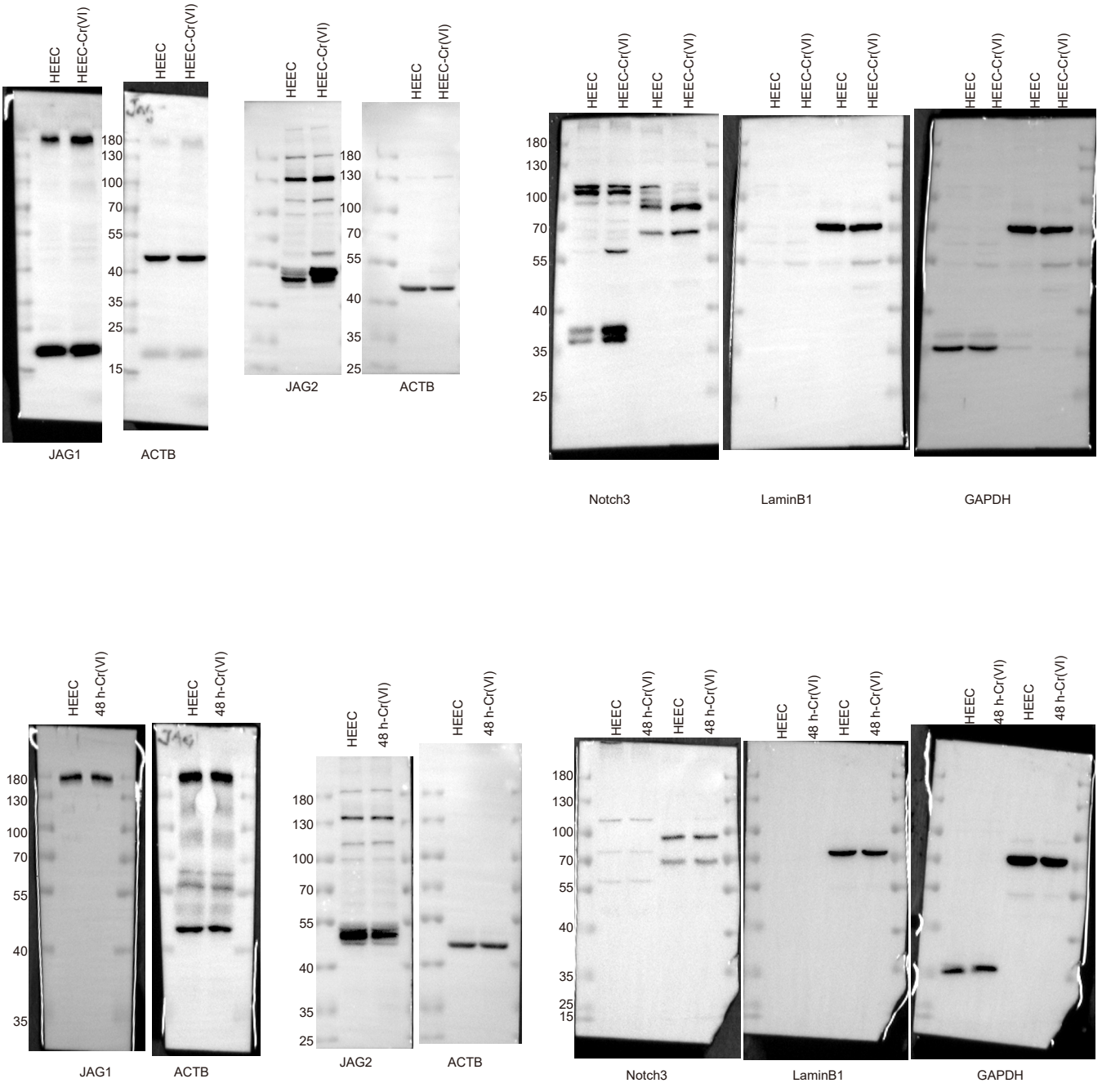


Fig4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HEEC-Cr(VI)
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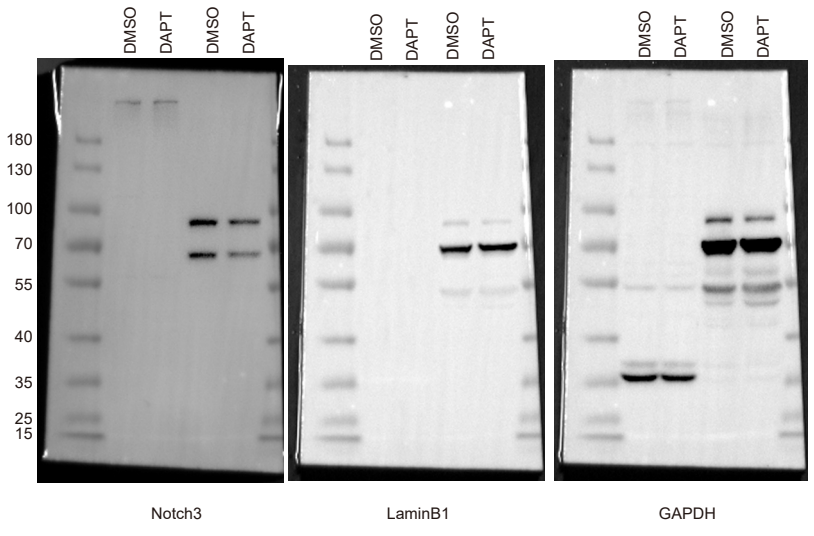
130
100
70
55
40
35
25
15
JAG1
ACTB
JAG2
Notch3
LaminB1
GAPDH
48 h-Cr(VI)

Fig5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DAPT
DMSO
DAPT
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100
70
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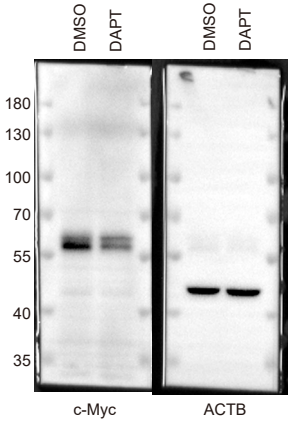
35
25
15
Notch3
LaminB1
GAPDH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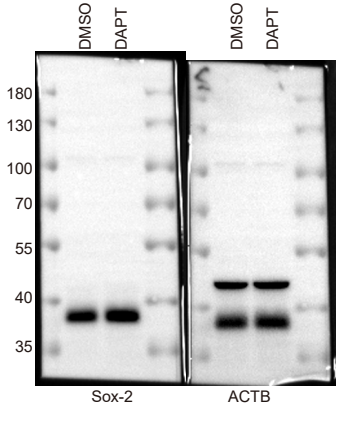
c-Myc
ACTB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Sox-2
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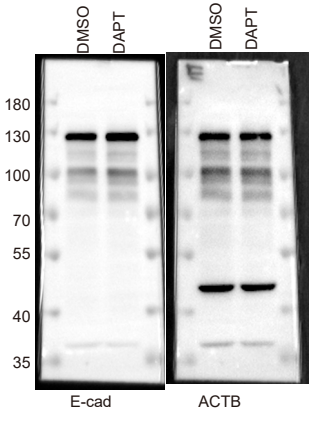
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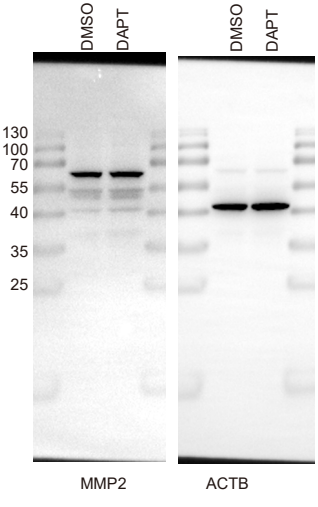
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